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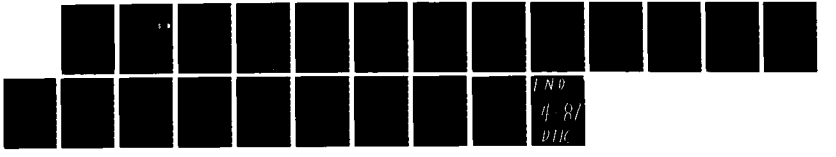

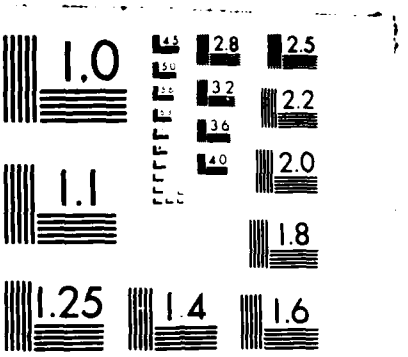

PROCEEDINGS

THE TWENTY-SIXTH MIDWINTER CONFERENCE OF IMMUNOLOGISTS

January 17-20, 1987
Asilomar Conference Center
800 Asilomar Avenue, Pacific Grove, California

TITLE: IMMUNOGLOBULINS AND DISEASE

CHAIRPERSONS: Dr. Roy Riblet, Medical Biology Institute, La Jolla, CA
Dr. Katherine Calame, University of California, Los Angeles, CA
Dr. Ruth Nussenzweig, New York University, New York, NY

SESSION I: STRUCTURE, RECOGNITION AND INTRACELLULAR TRAFFICKING OF MEMBRANE PROTEINS

Chairperson: DR. MATTHEW F. MESCHER
Medical Biology Institute, La Jolla, CA

Speakers:

DR. DON C. WILEY, Harvard University, Cambridge, MA
Structure of Membrane Glycoproteins Involved in Lymphocyte Recognition.

DR. PETER CRESSWELL, Duke University, Durham, NC
Intracellular Transport of HLA Antigens.

DR. MATTHEW F. MESCHER
Lymphocyte Recognition of Proteins on Model Membranes.

DR. VISHWANATH R. LINGAPPA, University of California, San Francisco, CA
Mechanisms of Membrane Protein Biogenesis

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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	2. GOVT ACCESSION NO. A177505
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Twenty-Sixth Midwinter Conference of Immunologists "Immunoglobulins and Disease"	5. TYPE OF REPORT & PERIOD COVERED Proceedings 11-01-86/10-31-87
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Submitted by Esther F. Hays, M.D. Executive Secretary	8. CONTRACT OR GRANT NUMBER(s) Office of Naval Research Award N00014-87-G-0003
9. PERFORMING ORGANIZATION NAME AND ADDRESS Regents of the University of California Dean's Office/School of Medicine 405 Hilgard Ave., Los Angeles, CA 90024	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS NR 441F718 AAN: 068342
11. CONTROLLING OFFICE NAME AND ADDRESS Scientific Officer, Cellular Biosystems Div. Office of Naval Research, 800 N. Quincy St. Arlington, VA 22217-5000	12. REPORT DATE 2-06-87
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) Office of Naval Research Resident Representative 1030 East Green Street Pasadena, CA 91106-2485	15. SECURITY CLASS. (of this report)
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution of this report is unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Distribution of this report is unlimited.

18. SUPPLEMENTARY NOTES

Presented at the 26th Midwinter Conference of Immunologists, Pacific Grove, California

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Immunology Conference, immunoglobulins

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Proceedings and abstracts of the 26th Midwinter Conference of Immunologists, held at Asilomar Conference Center, Pacific Grove, CA, January 17-20, 1987. The topic is "Immunoglobulins and disease."

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S/N 0102-LF-014-6601

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SESSION II: B CELL ACTIVATION AND DIFFERENTIATION

Chairperson: Dr. Robert L. Coffman, DNAX Research Institute of Molecular & Cellular Biology, Palo Alto, CA

Speakers:

DR. ROBERT L. COFFMAN
The Role of Lymphokines in Mediating Helper T Cell Function.

DR. ANTHONY S. FAUCI, National Institutes of Health, Bethesda, MD
Lymphokine-Mediated Immunoregulation of Human B Lymphocyte Function.

DR. DAVID C. PARKER, University of Massachusettes, Worchester, MA
T Cell Help for Antigen-Presenting B Cells.

DR. IRVING L. WEISSMAN, Stanford University, Stanford, CA
Early Lymphocyte Maturation.

SESSION III ANTIBODY EXPRESSION AND REGULATION

Chairperson: DR. KATHRYN CALAME, University of California, Los Angeles, CA

Speakers:

DR. FRED ALT, Columbia Physicians and Surgeons, New York, NY
Regulation of Gene Rearrangement Events During B-Cell Differentiation.

DR. TERESA IMANISHI-KARI, M.I.T., Cambridge, MA
Expression of Immunoglobulin Genes in Transgenic Mice

DR. TRISTRAM PARSLOW, University of California, San Francisco, CA
Regulatory Elements of the Immunoglobulin Kappa Gene

DR. KATHRYN CALAME, University of California, Los Angeles, CA
Regulation of Immunoglobulin Heavy Chain Gene Expression

SESSION IV ANTIBODY GENETICS AND MECHANISMS OF DISEASE

Chairperson: Dr. Roy Riblet, Medical Biology Institute, La Jolla, CA

Speakers:

DR. ROY RIBLET
The Organization of Antibody Genes.

DR. DENNIS CARSON, Research Institute of Scripps Clinic, La Jolla, CA
Rheumatoid Factors, Lymphoma, and Autoimmunity.

DR. MARK DAVIS, Stanford University, Stanford, CA
The xlr Gene Family and X-linked Immunodeficiency.

SESSION V IMMUNE RESPONSE TO PARASITES

Chairperson: Dr. Ruth Nussenzweig, New York University, New York, NY

Speakers:

DR. MARGARET PERKINS, Rockefeller University, New York, NY
Structure and organization of malarial (Merezoite) surface antigens.

DR. ALAN SHER, National Institutes of Health, Bethesda, MD
Evasion of the alternative complement pathway by Trypanosoma cruzi.

DR. DAVID SACKS, National Institutes of Health, Bethesda, MD
Induction of antiparasitic immune response to Leishmania using anti-idiotypic antibodies.

DR. RUTH NUSSENZWEIG
Anti-peptide immune response to sporozoite (malaria) antigens.

DR. BARRY BLOOM, Albert Einstein College of Medicine, Bronx, NY
Regulation of the spectrum of leprosy.

Anthony S. Fauci

ABSTRACT OF PRESENTATION
(200-250 words)

Factor-Induced Regulation of the Human B Cell Cycle. JL Ambrus, Jr, AS Fauci, NIAID, NIH, Bethesda, MD.

The human B cell cycle can be separated into stages of resting, activation, proliferation and differentiation. Activation normally occurs by crosslinkage of surface Ig by antigen or antigen equivalents such as anti-mu or Staphylococcus aureus Cowan I (SAC). Recent evidence has suggested that resting B cells can be activated by a soluble protein secreted by a T4+ lymphoma line which does not work via crosslinkage of surface Ig. In addition, resting B cells have receptors for interleukin-2 (IL-2) and B cell growth factor (BCGF). High concentrations of IL-2 can activate resting B cells, increase their expression of IL-2 receptors and induce them to proliferate. HMW-BCGF can act on resting B cells to upregulate the expression of c-myc although proliferation will only occur if an activation signal is then given.

Our analysis of B cell proliferation has centered around purification of HMW-BCGF, development of a monoclonal antibody to it, and identification of the receptor on B cells for HMW-BCGF with the use of a monoclonal antibody, BA5. The binding site is a 90 kd molecule which is expressed at very high levels by a cell line MB which was developed by long term culture of EBV negative B cells and then transforming them with EBV. The MB line has a high background proliferation which can be enhanced by the addition of HMW-BCGF. This line is being used to purify the protein recognized by BA5 for amino acid sequencing and ultimately cloning.

The monoclonal BA5 has also been used to study the abnormal regulation of expression of BCGF receptors in common variable immunodeficiency, SLE, and B cell neoplasms. Finally BA5 has been used to better understand the role of proliferation in B cell differentiation and immunoglobulin secretion. This work has increased our understanding of the complexity of human B cell physiology in normal and disease states.

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FROM: Anthony S. Fauci, M.D.

(Please enclose three to four references of your published work relevant to your talk).

RETURN TO: ESTHER F. HAYS, M.D.
Executive Secretary
Midwinter Conference of Immunologists
UCLA Warren Hall
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Los Angeles, California 90024

Intracellular Transport of HLA antigens

Peter Cresswell

Intracellular class II HLA antigens are associated with a transmembrane glycoprotein encoded by a non-HLA-linked gene. This glycoprotein, called the invariant chain, combines with the class II αβ dimer early in transport, probably in the rough endoplasmic reticulum, and traverses the Golgi apparatus in association with the class II complex. The invariant chain dissociates from the αβ dimer prior to class II antigen cell surface expression. Intracellular HLA-DR antigens have been isolated from B-lymphoblastoid cell lines treated with the ionophore monensin which blocks transport through the Golgi. These class II molecules contain α,β, and invariant chain subunits in association with a fourth molecule, identified as a chondroitin sulfate proteoglycan. The complex appears to be a tetramer containing one copy of each subunit, and is of the size of a 270,000 dalton globular protein as estimated by gel filtration. Work published by Sant, Cullen and Schwartz suggests that the proteoglycan is an alternatively processed form of the invariant chain. The kinetics of processing and cell surface expression of HLA-DR antigens argue that the class II molecules reside inside the cell for a considerable period of time (90-120 min) after traversing the Golgi apparatus. During this time they appear to be accessible to the endocytic recycling pathway followed by the transferrin receptor. This co-compartmentalization of an internalized ligand and newly-synthesized class II antigens is intriguing, and may be relevant to the phenomenon of antigen processing and presentation.

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Matthew F. Mescher

Lymphocyte Recognition of Proteins on Model Membranes

Much is known about the structures of the T cell and target cell proteins involved in antigen-specific recognition and transmembrane signaling. In contrast, relatively little is known regarding the detailed molecular interactions which occur, and the membrane parameters which influence and control these interactions. Artificial membranes bearing the appropriate target cell surface ligands provide a means of examining these aspects of T cell function. A recently developed artificial membrane system employing membranes supported on cell-size, silica-based beads is proving very effective in defining the molecular requirements for effective transmembrane signaling in precursor and effector cytolytic T lymphocytes. Class I MHC alloantigen on the bead-supported membranes is sufficient to stimulate responses of alloantigen-specific CTL. Recognition of alloantigenic determinants is required, and triggering is critically dependent on the density of alloantigen on the membrane. In addition, direct evidence has been obtained to show that recognition of conserved determinants on Class I molecules can augment the response. This augmentation is eliminated by addition of anti-Lyt 2 antibody, strongly suggesting that the conserved determinant recognition is mediated via Lyt-2/3. The Lyt-2/3 interaction leading to enhanced signaling does not appear to require that it bind to the same Class I molecule that is bound by the T cell receptor. The results obtained thus far have demonstrated that Class I alloantigen is both necessary and can be sufficient for specific binding and transmembrane signaling to occur. Interactions of CTL surface components with other target cell ligands may contribute to responses under some conditions (e.g. suboptimal Class I density) but are clearly not an absolute requirement.

RELEVANT REFERENCES:

Goldstein, S.A.N. and Mescher, M.F. (1986). Cell-size, supported artificial membranes (pseudocytes); response of precursor cytotoxic T lymphocytes to Class I proteins. J. Immunol. 137:3388.

Herrmann, S.H. and Mescher, M.F. (1986). The requirements for antigen multivalency in Class I antigen recognition and triggering of primed precursor cytolytic T lymphocytes. J. Immunol. 136:2816.

Burakoff, S.J. and Mescher, M.F. (1982). Reconstituted membranes and liposomes in the study of lymphocyte interactions. In Cell Surface Reviews (Poste, G. and Nicholson, G., eds.) North Holland: Elsevier.

Matthew F. Mescher

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Mechanisms of membrane protein biogenesis

Vishwanath R. Lingappa

Common and distinctive features of the biogenesis of different classes of newly synthesized integral transmembrane proteins have recently been elucidated. It appears that discrete sequences within the polypeptide chain are used in several ways to achieve variations on the theme of particular topologic fates, and thereby confer diverse transmembrane orientations in a programmed fashion. One "constitutive" function of such topogenic sequences is to serve as ligands for specific receptors in the membrane which direct the initiation or termination of translocation of nascent protein domains across the membrane of the endoplasmic reticulum. This has been demonstrated both by dissection of multiple topogenic sequences from the coding region for complex integral membrane proteins as well as by generation of complex artificial integral membrane proteins using coding regions from proteins of simpler transmembrane orientation. In the case of one particularly unusual protein, it appears that the function of topogenic sequences can be regulated co-translationally to achieve alternate integral membrane and secretory fates. In yet another case complex "higher order" processes appear to post-translationally modify the initial transmembrane orientation conferred by topogenic sequences, generating a secreted particulate form. Here I will summarize current thoughts on some of these general and special events in membrane protein biogenesis and discuss ongoing experimental approaches to elucidation of their mechanism.

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THE ROLE OF LYMPHOKINES IN MEDIATING HELPER T CELL FUNCTION

Robert L. Coffman, DNAX Research Institute
901 California Avenue, Palo Alto, Ca. 94304

Mouse helper T (Th) cells can be divided into two major subsets based on the production of lymphokines which affect B cells. One subset (Th1) produces IL-2 and IFN-γ but produces no IL-4 (BSF-1). The second subset (Th2) produces IL-4 but no IL-2 or IFN-γ. We have been attempting to learn the roles each of these lymphokines play in the growth and differentiation of B cells and in the regulation of expression of the different immunoglobulin isotypes. This talk will focus on the regulation of IgE expression by IL-4 and IFN-γ and the regulation of IgA expression by a recently discovered IgA-enhancing activity.

IL-4 can stimulate both B cell growth and differentiation, but its most striking effect is its 100 to 1000-fold enhancement of IgE production in both Th-dependent and Th-independent B cell cultures. The observations that all T_H clones which can provide help for an IgE response belong to the IL-4-producing Th2 subset and that anti-IL-4 antibodies can inhibit this IgE helper function provide further evidence that IgE production requires IL-4.

IFN-γ can totally inhibit the effects of IL-4 on B cells, including the IL-4-mediated expression of IgE. The potent but opposing effects of IL-4 and IFN-γ on the IgE response suggest that the magnitude of an IgE response may depend upon the relative levels of these two lymphokines, and thus, upon the relative state of activation of the two Th subsets which produce them.

We have recently isolated and characterized another lymphokine expressed predominantly by Th2 clones. Like IL-4, this molecule stimulates both B cell growth and differentiation, but, in contrast to IL-4, specifically enhances IgA production by B cells. We have isolated a cDNA clone encoding this molecule and have found it to be identical to IL-5 (also called BCGF-2 or TRF-1).

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Mosmann, T.; Cherwinski, H.; Bond, M.W.; Geidlin, M.A. and Coffman, R.L. Two Types of Mouse Helper T cell clones: Definition According to Profiles of Lymhokine Activities and Secreted Proteins. J.Immunol. 136, 2348-2357, 1986.

David C. Parker

T Cell Help for Antigen-Presenting B Cells

Most immunologists would agree that B cells get help by presenting antigen to helper T cells. Experiments from several laboratories including our own have shown that B cells are remarkably efficient antigen-presenting cells for antigen initially bound to membrane Ig, the antigen receptor of the B cell. Using rabbit anti-mouse Ig in place of antigen, we have shown that membrane Ig-mediated antigen presentation by small B cells to rabbit globulin-specific T cell lines results in a vigorous polyclonal antibody response. As shown with other antigen-presenting cells, antigen presentation by small B cells requires an antigen processing step; in small B cells, this step takes 4 to 8 hours and is sensitive to chloroquine and radiation (2000 rads). Antigen is no longer associated with membrane Ig at the time of antigen presentation, so antigen recognition by B cell and T cell is sequential rather than simultaneous. The polyclonal antibody response which follows MHC-restricted helper T cell recognition of processed antigen on the B cell surface occurs in the absence of other kinds of antigen-presenting or accessory cells, and does not require a signal delivered through membrane Ig to the B cell, since monovalent Fab fragments of anti-Ig, which do not activate B cells, are just as effective as divalent $F(ab')_2$ fragments. Once the T cell is activated, help is delivered preferentially to the activating B cell. However, when the T cells are optimally activated, then bystander small B cells without antigen or with inappropriate MHC alleles do proliferate well. The delivery of this bystander help is short range, since it does not occur across a Nucleopore membrane which allows passage of stable lymphokines. It does not appear to involve class II MHC molecules as signal transducers, since it is insensitive to blocking concentrations of antibodies against bystander class II alleles.

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D. C. Parker, E. J. Gosselin, B. J. Beaudoin, and H.-P. Tony. In Progress in Immunology VI. Academic Press, in press 1987.

Control of Genomic Rearrangement Events during Lymphocyte Differentiation

Frederick W. Alt, Stuart Lutzker, Keith Blackwell, and George Yancopoulos
Department of Biochemistry, College of Physicians and Surgeons of Columbia University, New York, New York 10032

The genes which encode the variable region of antigen receptor chains are assembled from multiple germline DNA elements. We provide evidence that a common recombinase is involved in the assembly of immunoglobulin heavy and light chain variable region gene segments and T cell receptor variable region gene segments. The assembly of these different types of gene segments is controlled in a tissue- and stage-specific fashion as well as with respect to allelic exclusion. In this context, the specificity of the common recombinase appears to be controlled by modulating the "accessiblity" of the substrate gene segments. We have undertaken of preliminary molecular definition of "accessibility" through the transfer of various types of variable region gene recombination substrates into Abelson murine leukemia virus-transformed pre-B cell lines which actively assemble endogenous heavy chain variable region genes. The results of these experiments suggest that DNAase sensitivity as conferred by the heavy chain enhancer element may be necessary but is not sufficient to confer accessiblity of introduced substrates to recombinase; such accessibility appears to be more intimately associated with transcription of the targeted gene segments.

Additional support for accessibility, and more specifically, transcription, in targeting rearrangement events during B cell differentiation comes from our studies of heavy chain class switching in Abelson virus transformed pre-B cell lines. Class switching involves a different type of recombination event which switches the expressed heavy chain constant region with a downstream constant region through a recombination/deletion mechanism. We have found that switches between an expressed constant region gene and a downstream constant region are preceded by transcription of the two constant region genes. The transcripts are intiated from promoters which lie upstream of each of the involved constant region genes and which generate germline transcripts which cannot encode proteins. The latter observation is consistent with a specific role for transcription of these regions with respect to the class switch recombination event.

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REGULATORY ELEMENTS OF THE IMMUNOGLOBULIN KAPPA GENE

Tristram G. Parslow, M.D., Ph.D.
Depts. of Pathology and of Microbiology and Immunology
University of California, San Francisco

At least two distinct DNA sequence elements have been found to influence the transcription of immunoglobulin kappa genes. One of these is a tissue-specific transcriptional enhancer; the other is an octanucleotide sequence motif (ATTTGCAT) found at a stringently conserved position in the promoters of all known light chain genes. We have recently found that this same octanucleotide is also an essential component of the thymidine kinase (tk) promoter of Herpes simplex virus, and have used this promoter to evaluate the role of the octanucleotide in transcriptional regulation, particularly with regard to its interactions with promoter and enhancer elements. The presence of the octanucleotide increases tk promoter activity in both lymphoid and non-lymphoid cells without altering the initiation site. The action of the kappa enhancer augments that of the octanucleotide multiplicatively; when both elements are present, tk promoter activity in B lymphoid cells is increased by more than an order of magnitude. In contrast, the presence of the octanucleotide in this promoter markedly reduces its response to a non-immunoglobulin enhancer, suggesting that the octanucleotide may mediate a selective interaction among promoters and enhancers.

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Kathryn Calame

TRANSCRIPTIONAL REGULATION OF IMMUNOGLOBULIN HEAVY CHAIN GENES

C. Peterson, X. Wang, S. Eaton and K. Calame

We are studying the DNA sequences and DNA-binding proteins which are required for the activity of the immunoglobulin heavy chain transcriptional promoter and enhancer elements.

Previous work from our laboratory has demonstrated that the IgH enhancer region contains at least seven sites where nuclear proteins bind in vitro; a minimum of four different proteins bind to these sites. We are using two approaches to study the mechanism of this complex regulatory element further. First, we have begun purification of the enhancer-binding proteins. We have purified three proteins sufficiently to allow precise localization of their binding sites by methylation interference and DNase I footprinting studies. One protein, IgHE III, which appears to bind only to the heavy chain enhancer but not to the SV40 or kappa enhancers has been purified several thousand fold and appears to be about 60kD in size. Our second approach to the detailed study of enhancer mechanism has been to create small deletion mutations in the sites identified by protein binding in vitro and to test them in vivo for enhancer function. Analysis of several such mutants establishes the functional importance of these enhancer-binding proteins and is allowing us to ascertain which proteins may interact with one another.

In order to study the DNA elements which are functionally important for a Vh gene promoter region, we have constructed several deletion and base change mutations in the promoter for the V1 heavy chain gene and have tested the activity of the deleted promoters by transfection into plasmacytoma cells. Our results confirm those of Neuberger showing a requirement for a conserved octamer sequence. In addition, we find other sequences upstream of the octamer which are required for normal transcription from this promoter. Our current goal is to determine if any of these elements are involved in the B-cell specificity of the promoter and to characterize the proteins which recognize them.

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RHEUMATOID FACTORS, LYMPHOMA, AND AUTOIMMUNITY

Dennis A. Carson, M.D.

Rheumatoid factors are autoantibodies against IgG, that were defined originally by the ability to react with antigenic determinants in the Fc region. Rheumatoid factors represent a normal component of the immune network. The autoantibodies promote complement fixation and immune complex clearance. They amplify the avidity of polyclonally induced IgG. Genes related to the primary structure of rheumatoid factor light chains are widely distributed in the human population, and have been conserved during the evolution and dispersion of the species. Products of these genes may be detected with anti-idiotypic antibodies against synthetic peptides corresponding to individual hypervariable regions on rheumatoid factor light chains. Such anti-peptide antibodies provide unique reagents for analyzing the genetics of immunoglobulins in outbred populations. Rheumatoid factor precursors are abundant among immature B lymphocytes. Some of these cells may tend to localize to mucosal surfaces, where they are stimulated directly by pathogenic microorganisms with polyclonal B cell activating properties. Rheumatoid factor synthesis regularly accompanies all secondary immune responses, but is usually transient. Production of the autoantibody is T cell dependent. The T cells may recognize antigen in an IgG-antigen immune complex that is processed and presented by rheumatoid factor B cell precursors. Rheumatoid factor associated light chain idiotypes are rare in serum IgG, and on IgG myeloma proteins. They are common among monoclonal IgM proteins, and on the surface of the malignant B cells from patients with chronic lymphatic leukemia. The rheumatoid factors that are produced by patients with mixed cryoglobulinemia, hypergammaglobulinemic purpura, and primary Sjogren's syndrome, can share idiotypic antigens with monoclonal rheumatoid factors. Rheumatoid factor synthesis in the three diseases may reflect an abnormal B cell proliferation that is not antigen-driven, and that can degenerate into malignancy. The rheumatoid factors in patients with rheumatoid arthritis are diverse, and almost certainly represent the outcome of antigen-induced, T cell dependent mechanisms. The antigens that drive the T cells have not been identified, but could represent exogenous microorganisms, self-components, or idiotypic antigens that fortuitously interact with rheumatoid factors.

Structure of *Plasmodium falciparum* Merozoite Surface Antigens

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The merozoite, the extracellular form of the malarial parasite of the asexual, blood stage is unicellular and surrounded by a double plasma membrane and thick surface coat. Unlike the sporozoite, which is composed of a single antigen, the CSP, the merozoite surface is antigenically complex. In *Plasmodium falciparum*, work in many laboratories has identified at least six distinct proteins localized on the surface. All these proteins are recognized by hyper-immune serum from infected humans and since they represent targets of immune attack the structure of the important epitopes is of considerable interest. The major merozoite protein is one of m.w. 200,000. This protein which exhibits limited size and antigenic diversity between different isolates is a glycoprotein. At the merozoite stage it is processed to polypeptides of smaller size, 85,000, 50,000 and 28,000 m.w. It is associated with the plasma membrane and the 50,000 m.w. processed polypeptide remains attached to the merozoite during reinvasion into the host erythrocyte. A second glycoprotein of 56,000 m.w. has also been localized to the merozoite surface. Four other surface proteins, ranging in size from 140,000 m.w. to 75,000 m.w. have also been identified. These proteins are soluble, do not appear to be associated with the plasma membrane but are located on the surface coat. They all appear to be readily shed from the merozoite prior to or during invasion into the erythrocyte. One of the soluble proteins of m.w. 130,000 has been cloned and sequenced. The 3' end of the molecule consists of 11 repeats of a highly conserved sequence of 50 amino acids. The repeat domain constitutes the only epitope recognized by human immune sera. The 130,000 m.w. protein appears to bind to the erythrocyte receptor, glycophorin. It has been shown to be conserved antigenically in 12 geographic isolates of *P. falciparum*.

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Alan Sher

EVASION OF THE ALTERNATIVE COMPLEMENT PATHWAY BY TRYPANOSOMA CRUZI. Alan Sher and Keith Joiner, Laboratories of Parasitic Disease and Clinical Investigation, National Institute of Allergy and Infectious Disease, NIH, Bethesda, MD 20892

Before entering the definitive host from their invertebrate vectors, protozoan parasites undergo a series of adaptive changes which enable them to evade vertebrate immunologic defenses. We have been studying the developmental adaptation of Trypanosoma cruzi (the causative agent of Chagas' Disease) to the alternative complement pathway (ACP), an important mechanism of natural resistance to infection. The insect vector stage of the life cycle or epimastigote (EPI) is highly sensitive to ACP mediated lysis in fresh normal serum. Prior to infecting the vertebrate host, the parasite transforms into a metacylic trypomastigote which fails to activate the alternative complement pathway and is therefore resistant to serum lysis. We have been investigating the mechanism underlying this host pre-adaptation in metacyclic trypomastigotes (CMT) derived from EPI in stationary culture. Studies with purified complement proteins indicated that CMT fail to activate the ACP because C3b on the parasite surface is unable to bind factor B with high affinity thereby preventing amplification. We have identified a series of developmentally regulated changes in the parasite which may result in this alteration in C3b-B interaction. These include: (a) the synthesis by CMT of a 90-115 kd doublet which when removed from the surface reverts the parasite to an ACP activator; (b) alterations in the expression of a 72kd glycoprotein which is the acceptor molecule for C3b on EPI; (c) the stage specific synthesis of a soluble decay accelerating activity (DAF) which destabilizes the interaction of B and C3b on CMT. Our current understanding of these different mechanisms and their functional interrelationships will be discussed.

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INDUCTION OF CARBOHYDRATE SPECIFIC ANTI-PARASITE RESPONSES USING ANTI-IDIOTYPIC ANTIBODIES

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The induction of immune responses directed against carbohydrate epitopes may be particularly important with respect to immunity against parasitic protozoa and helminths. Because carbohydrate antigens cannot be easily reproduced synthetically or by recombinant DNA technologies, we have studied the use of anti-idiotypic (anti-Id) antibodies to induce immune responses against these determinants. A monoclonal mouse antibody (29.26) recognizes an unusual carbohydrate epitope on the 72K glycoprotein, which is the major cell surface antigen found on invertebrate stages of Trypanosoma cruzi. Immunization of BALB/c mice with affinity-purified rabbit anti-29.26 anti-Id induced high titers of antibodies against the 72K glycoproteins which were reactive with the surface of T. cruzi epimastigotes. When assayed on a strain of T. cruzi which contains the 72K glycoprotein, but lacks exposed carbohydrate epitope recognized by 29.26, no binding was observed, suggesting that the antibody induced by the anti-Id was specific for a carbohydrate determinant. The induction of specific antibodies against 72K in both rabbits and guinea pigs following immunization with anti-Id suggests either the 29.26 Id represents an interspecies cross-reactive Id, or the presence of an internal image of the carbohydrate epitope within the anti-Id antibody population.

We have also been able to induce immunity in mice against cutaneous leishmaniasis by immunizing with anti-Id raised against a monoclonal antibody (79.3) with specificity for a leishmanial lipopolysaccharide (L-LPS). This molecule is found on the cell surface of promastigotes and on the surface of infected macrophages. It is thought to be required for attachment to macrophages and for intracellular survival. Mice immunized with 79.3 affinity purified L-LPS were immune to fatal leishmaniasis. Immunization of BALB/c mice with affinity purified rabbit anti-79.3 anti-Id also protected these mice against progressive disease. While antibodies against L-LPS could be detected in anti-Id treated mice, since immunity to leishmanial infection is dependent upon cell-mediated immune responses, we presume that the anti-Id also activated T-cell responses. The effect of anti-Id on T-cell responses, and in particular on responses directed against non-protein determinants, is being investigated further.

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Barry R. Bloom

IMMUNOREGULATION IN LEPROSY

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The various forms of leprosy form a clinical and immunological spectrum which offers extraordinary possibilities for insight into immunoregulatory mechanisms in man. In tuberculoid leprosy, patients develop high levels of cell-mediated immunity which ultimately results in killing of bacilli in the tissues, albeit often with damage to nerves. At the lepromatous pole, patients exhibit selective immunological unresponsiveness to antigens of M. leprae. In vitro experiments suggest that lepromin-induced suppressor cells, exist in blood and in lesions which are $T8^+$ and express the activation markers, HLA-DR and FcR. The one known unique species of antigen of the leprosy bacillus is a phenolic glycolipid, and it appears that many T_S cells recognize the terminal trisaccharide of this unique antigen. Depletion of the T_S cells restores in vitro reactivity of lymphocytes to lepromin in about a third of lepromatous patients, and the T8 suppressor cells disappear after successful immunotherapy. It has been possible to establish T8 lines and single cell clones from lesions of patients with lepromatous leprosy. These clones have the capability, when stimulated by M. leprae antigens, of suppressing mitogen responses of peripheral blood cells or specific antigen responses of T4 cell clones in an MHC Class II restricted fashion.

The existence of mAb and T cell clones permits screening of DNA expression libraries for antigens that might be protective, and which might be introduced into BCG to produce a recombinant mycobacterial vaccine. Initial experiments in introducing foreign DNA into mycobacteria will be discussed.

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ABSTRACT OF PRESENTATION
(200-250 words)

Anti-peptide immune response to sporozoite (malaria) antigens

Ruth S. Nussenzweig

Immunity to the sporozoite stage of malaria parasites is T cell dependent and involves both antibody and cell mediated responses directed against a highly immunogenic surface protein, the circumsporozoite (CS) antigen (1,2). The primary structure of this protein is similar in all malarial species, and contains an immunodominant domain of tandemly repeated sequences of 4 to 12 amino acid each. We have investigated the antigencity of these repeats and of a yeast derived recombinant CS protein of the human malaria parasite P. vivax, and also attempted to define the T epitope(s) of this antigens.

The repeat domain of this protein contains 19 repeats of a nine amino acid sequence, occurring as 2 variants which differs in one of their amino acids. This raised the question of whether this variation would generate epitope diversity reflected in the fine specificity of mouse monoclonal and human antibodies to this CS protein.

The results of inhibition of binding immuno-assays revealed the presence of at least 2 repeated overlapping epitopes. They also indicated that human immune sera contain antibodies which recognize additional epitopes in the recombinant CS protein, not represented by the repeat peptides.

Using congenic mice immunized with the recombinant P. vivax CS protein, or with a synthetic peptide corresponding to two nonamer repeats, we found that the T cell response is genetically restricted to the H-2^K haplotype.

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